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Disposal of ferritin in the glomerular mesangium of rats

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Disposal of ferritin in the glomerular mesangium of rats. Mechanisms involved in the mesangial disposal of exogenous macromolecules were investigated in rats using native horse-spleen ferritin as a probe. After intravenous injection of ferritin into rats, renal tissue was examined at intervals from 1 min to 28 days. Immunofluorescence microscopy studies showed that the biodegradable protein component, apoferritin, was detectable in glomeruli within 10 min, was most prominent by 6 hr, and became undetectable at 14 to 28 days. By contrast, glomerular staining for the iron core of ferritin was strongest at 24 hr and did not noticeably change during the remaining study period. Apoferritin and iron were localized in a mesangial pattern including the stalk and lacis area. Transmission electron microscopy showed entry of ferritin into the mesangial matrix within 10 min and incorporation into cells in the mesangium by 30 min. After day 3, all ferritin was found intracellularly. No shift of ferritin from the glomerular tuft to the vascular pole was observed and ferritin was not found in adjacent interstitial areas or macula densa cells. Counts of mesangial Ia-antigen bearing cells, demonstrated in cryostat sections by immunofluorescence, increased from a mean of 2.1 per glomerular tuft section (range, 0 to 6), in noninjected rats, to 2.8 (range, 0 to 8) 14 days after ferritin injection. However, simultaneous phase contrast microscopy revealed that only a proportion of Ia-positive cells was associated with mesangial ferritin granules and that the bulk of ferritin was located to Ia-negative cells. Counts of infiltrating monocytes, monitored by histochemical staining for nonspecific esterase, rose from 0.1 stained cells per tuft section in controls to a maximum of 1.3 (range, 0 to 3) on day 3, at a time when most cells in the mesangium showed ferritin uptake by electron microscopy. The results demonstrate that the glomerulus disposes of ferritin, upon entry into the mesangial space, by endocytosis by mesangial cells involving degradation of apoferritin and storage of iron cores in phagolysosomes. The great majority of these cells do not bear Ia-antigens. The contribution of infiltrating monocytes to this process appears small. While the fraction of ferritin which is removed from the mesangium by an extracellular route remains undetermined, there is no appreciable egress of ferritin from the glomerulus via the vascular pole to the adjacent interstitium or tubules.

Elimination de la ferritine dans le mésangium glomérulaire de rats. Les mécanismes impliqués dans l'élimination mésangiale de macromolécules exogènes ont été étudiés chez des rats en utilisant de la ferritine native de rate de cheval. Après injection intraveineuse de ferritine aux rats, le tissu rénal était examiné à des intervalles compris entre 1 min et 28 jours. Les études en microscopie avec immunofluorescence ont montré que le composant protéique biodégradable, l'apoferritine, était détectable dans les glomérules en 10 min, qu'il était prédominant en 6 hr et devenait indétectable de 14 à 28 jours. Par contraste, la coloration glomérulaire pour le noyau ferrique de la ferritine était très forte à 24 hr et ne changeait pas de façon notable pendant le reste de la période d'étude. L'apoferritine et le fer étaient localisés dans un site mésangial comprenant la tige et l'aire du lacis. La microscopie électronique par transmission a montré l'entrée de ferritine dans la matrice mésangiale en 10 min et l'incorporation dans les cellules du mésangium en 30 min. Après le jour 3, toute la ferritine était trouvée intracellulaire. Aucun déplacement de la ferritine de la touffe glomérulaire au pôle vasculaire n'a été observé, et la ferritine n'était pas trouvée dans les aires interstitielles adjacentes, ni dans les cellules de la macula densa. Le compte des cellules mésangiales comportant de l'antigène Ia, mises en évidence dans des sections au cryostat par immunofluorescence s'élevaient d'une moyenne de 2,1 par section de touffe glomérulaire (intervalles zéro à 6) chez les rats non injectés, à 2,8 (intervalle zéro à 8) 14 jours après l'injection de ferritine. Cependant, la microscopie en contraste de phase simultanée a révélé que seulement une proportion de cellules Ia positives était associée avec des granules mésangiaux de ferritine et que la majorité de la ferritine était localisée dans des cellules Ia négatives. Le compte des monocytes infiltrants, répérés par coloration histochimique pour l'estérase non spécifique, se sont élevés de 0,1 cellules côlorées par section de touffe dans les contrôles, un maximum de 1,3 (intervalle de zéro à 3) au jour 3, à un moment où la plupart des cellules du mésangium montraient une captation de la ferritine par microscopie électronique. Ces résultats démontrent que le glomérule se débarrasse de la ferritine lors de son entrée dans l'espace mésangial, par endocytose par les cellules mésangiales mettant en jeu une dégradation de l'apo-ferritine et un stockage des noyaux ferriques dans des phagolysosomes. La grande majorité de ces cellules n'a pas d'antigène Ia. La contribution des monocytes infiltrants à ce processus apparaît faible. Bien que la fraction de ferritine enlevée à partir du mésangium par une voie extracellulaire reste indéterminée, il n'y a pas de sortie appréciable de ferritine du glomérule par le pôle vasculaire vers l'interstitium ou les tubules adjacents.

Multiple experimental studies have shown that the glomerular mesangium plays a prominent role for the disposal of immune complexes, protein aggregates, and inert macromolecular probes after these are deposited in various sites of the glomerular tuft (reviewed in [1–3]). The mechanisms thought to be operative in this elimination process include extracellular passage of the macromolecules through mesangial matrix channels and uptake and degradation or storage by cells resident in the mesangial space [3]. Besides the predominant intrinsic smooth-muscle type mesangial cells, a small subpopulation of Ia-antigen bearing, bone-marrow derived cells has been identified recently in the mesangium of healthy rats [4, 5]. In those studies, Schreiner et al [4] reported that a major fraction of these immune competent cells possesses endocytic abilities in vivo and in vitro. This finding raised the question about the contribution of resident Ia-antigen bearing cells to the over-all mesangial uptake of deposited macromolecules. Another question in this context concerns the involvement of blood-borne monocyte-macrophages in the process of mesangial deposit removal. While the disposal of various protein and nonprotein tracers has been demonstrated to proceed without conspicuous monocyte participation [6–9], it has also been observed that

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certain mesangial probes induce prominent monocytic infiltration of the glomeruli [10, 11]. It is likely that the nature and the biologic activity of the deposits are largely responsible for the extent of participation of inflammatory cells. It is noteworthy, however, that the predominantly mesangial localization of endogenous immune complexes and complement components, seen in various experimental and human glomerular diseases, is frequently not associated with a sizeable monocytic contribution [12–15].

Among the macromolecular protein probes which are available to examine the mesangial disposal process without inducing prominent inflammatory or chemotactic stimuli, heterologous ferritin, as originally introduced by Farquhar and Palade [6] and Farquhar, Wissig, and Palade [16], has the advantage to be homogenous as to size and charge and to allow simultaneous assessment of the fate of the degradable protein portion, apoferritin, and of the inert iron core which facilitates identification of the ferritin molecules regardless of the integrity of its proteinaceous shell. These properties help to overcome the drawback of all-protein probes which are ordinarily visualized by immunohistological techniques and, thus, require completely intact antigen characteristics for detection. In addition, use of ferritin yields more information than of inert probes, such as colloidal carbon or iron dextran, because these lack a digestible component of protein and are, thus, inadequate to examine questions concerning glomerular proteolytic capacities.

In this study, the mesangial processing of ferritin was examined in rats with regard to time course and route of removal as well as to the contribution of Ia-antigen bearing cells and infiltrating mononuclear phagocytes.

Methods

Animals. Forty-eight male Sprague-Dawley rats with initial weights of 150 g and free access to food and water were used in the experiments.

Ferritin preparation. Horse-spleen ferritin was obtained from Accurate Chemical and Scientific Corp., Westbury, New York. To eliminate traces of cadmium, this preparation was dialyzed for 24 hr against EDTA in 0.07 M phosphate-buffered saline (PBS), pH 7.2, followed by dialysis against PBS alone for 48 hr. The resulting preparation was stored under sterile conditions at 4°C and centrifuged at $\times 5000g$ before use to eliminate any large ferritin aggregates formed. The intravenous injection of this preparation was tolerated well by the animals.

Experimental procedures. Thirty-six rats received 50 mg of ferritin/100 g body weight by tail vein injection under light ether anesthesia. Serum was obtained serially in representative animals for determination of ferritin levels by single radial immunodiffusion [17] using a specific rabbit-derived antiferritin IgG (Accurate Chemical and Scientific Corporation). The specificity of the antiserum was shown using the double immunodiffusion technique, by demonstrating single precipitation lines against the employed ferritin preparation and against another horse-spleen ferritin preparation kindly provided by Drs. A. Vogt and S. Batsford, University of Freiburg, Federal Republic of Germany [18]. At selected time intervals, from 1 min to 28 days after ferritin injection, the rats were sacrificed in groups of two to four. Twelve noninjected rats served as controls. Left and right kidneys were ligated at the hilus and removed before exsanguination of the animals.

Tissue processing

Immunofluorescence microscopy. Half of one kidney was snap-frozen in liquid nitrogen. Frozen tissue samples were used for indirect fluorescence studies which were carried out on 5-μm cryostat sections. Rabbit antisera against ferritin and rat IgG were used in combination with FITC-conjugated anti-rabbit IgG (Cappel Laboratories, Cochranville, Pennsylvania). Incubation of renal sections from eight untreated control rats with these antisera, individually or in combination, caused no or faint focal glomerular fluorescence. Preabsorption of the anti-ferritin serum with two preparations of purified horse-spleen ferritin (see above) abolished the glomerular staining noted in renal sections of ferritin-treated rats when nonpreabsorbed antiserum was used. Semiquantitative grading of glomerular ferritin deposition was performed on a scale from 0 to 4+ following the criteria of Takamiya et al [18]. Each section was scored blindly, and the mean score was used. Simultaneous evaluation by phase contrast microscopy served to visualize the glomerular histology and to identify the dense granules of ferritin. Evaluation of the stained sections was performed with an incident light fluorescence photomicroscope with phase contrast equipment (Dialux 20, Leitz, Rockleigh, New York).

Ia-positive cells. These cells were identified in 6- to 8 μm-thick, frozen sections, from eight untreated control rats and from 14 rats injected with ferritin. Sections were incubated with mouse-derived monoclonal antibody against rat Ia-antigen and stained with FITC-conjugated rabbit anti-mouse IgG (Accurate Chemical and Scientific Corporation). The anti-rat Ia-antibody was the mouse IgG preparation MAS 043c, studied in detail by McMaster and Williams showing binding to rat Ia-antigens which correlate with the Ia-specificity 17 or 18 in the mouse [19]. In preliminary studies we demonstrated that this antibody stained individual cells in the interstitial connective tissue of liver and kidney as well as cells in glomeruli. The results of the fluorescence studies for Ia-bearing cells in rat kidneys, obtained with that antibody, were confirmed by using mouse-derived antibodies directed against mouse Ia 1, 2, 3, 7 characterized and kindly provided by Dr. John G. Ray, National Institute of Health, Bethesda, Maryland (Sterzel, Perfetto, unpublished observations). Incubation of renal sections from control rats and ferritin-treated rats with normal mouse IgG instead of anti-Ia antibody caused no or only mild background fluorescence of renal tissue. Mean counts of Ia-positive cells in glomerular tufts were obtained from 50 glomeruli per rat.

Iron stain. The other half of the kidney was fixed in Bouin's solution and, after paraffin-embedding, sections were stained with hematoxylin and eosin and Prussian blue. Glomerular iron deposits were scored on a 0 to 4+ scale. Few iron-stain positive granules in only a portion of glomeruli were called +/−. Ratings of 1+ to 4+ implied that all glomeruli were positive; 1+ indicated that weakly stained granules were present in parts of glomeruli. Moderately strong iron staining in most portions of glomerular tufts was rated 2+. Strong and uniform staining was 3+, and extensive staining of all glomerular lobules was 4+. Particular attention was paid to the distribution of iron deposits in the periphery and the stalk of the glomerular tuft, in the lacis area and the adjacent cortical interstitium as well as tubules.

Electron microscopy. One-millimeter blocks of cortical tissue of the other kidney were fixed for 4 hr at 4°C in 4% formaldehyde-5% glutaraldehyde fixative in 0.2 M cacodylate buffer, pH

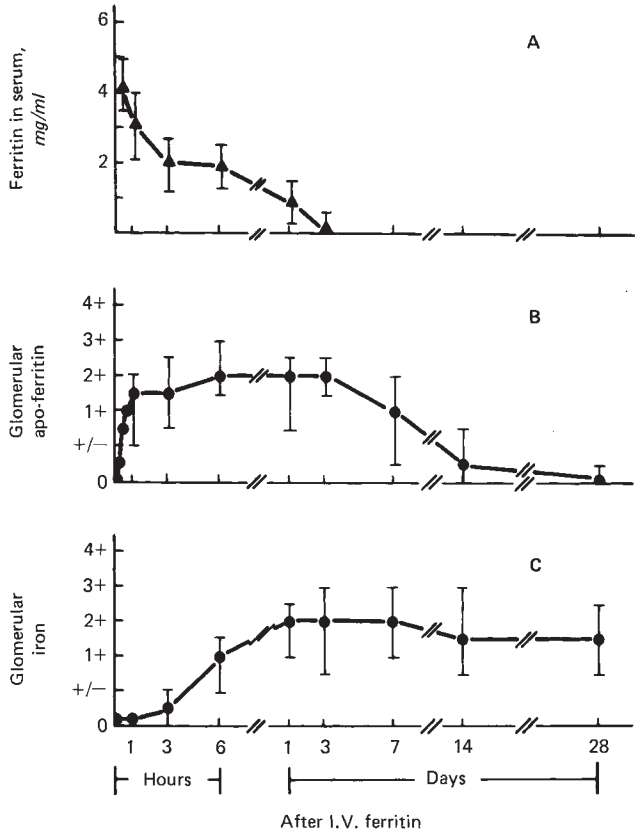

Fig. 1. A *Time course of ferritin concentration (mean ± SEM) in serum after intravenous injection; N = 4.* **B** *Time course of glomerular staining for apoferritin by indirect immunofluorescence.* Each *point* represents mean result (± range) obtained from 3 to 6 rats. **C** *Time course of glomerular staining for iron by light microscopy.* Each *point* represents mean results (± range) obtained from 3 to 6 rats.

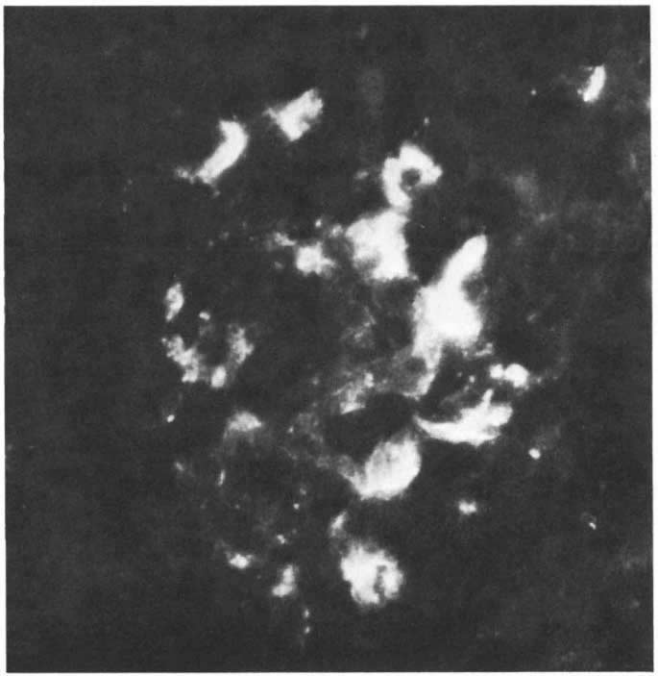

Fig. 2. *Immunofluorescence staining for apoferritin in renal tissue of a rat 6 hr after ferritin administration.* Glomerulus shows granular staining in a mesangial distribution. (×650)

7.2. After washing in buffer, the tissue was processed for electron microscopy as described previously [13]. Thin sections were stained with uranyl acetate-lead citrate or bismuth subnitrate [20]. Location of intact ferritin or its iron cores was determined and their amounts were rated on a 0 to 3+ scale (0 = negative; +/− = trace; 1+ = moderate; 2+ = heavy; 3+ = massive amounts of ferritin present).

Histochemistry for nonspecific esterase. Samples of the remaining renal tissue of 12 untreated control rats and 32 ferritin-injected rats were fixed in buffered formaldehyde acetone, pH 6.6, washed in Holt's sucrose-gum acacia buffer for 24 hr and then snap-frozen. Cryostat sections, 5-μm thick, were then stained for nonspecific esterase, using α-naphthyl butyrate as substrate, following a modification of the method of Yam, Li, and Crosby [21], as described previously [13]. Negative controls consisted of incubations in medium containing 1 μg/ml sodium fluoride (NaF), which inhibits the histochemical reaction for nonspecific esterase of monocytes-macrophages. Mean counts of esterase-positive cells in glomerular tufts were obtained, evaluating a minimum of 50 glomeruli of all sizes in each rat. In selected rats, sections were first stained for nonspecific esterase and then stained for iron to relate the presence of esterase-positive cells to the distribution of ferritin. In preliminary studies it was shown that the iron-staining procedure did not abolish positive staining for esterase.

Results

Immunofluorescence microscopy. After intravenous injection of 50 mg ferritin/100 g body weight, the serum concentrations of ferritin fell rapidly over the first 3 hr, then declined more gradually and ferritin was no longer detectable by day 3 (Fig. 1**A**). The time course of the glomerular fluorescence findings for ferritin is shown in Figure 1**B**. Maximal fluorescence was noted within 1 to 6 hr after ferritin injection. At this time all glomeruli were involved, most in a diffuse, some in a segmental fashion. The staining pattern was always granular showing a mesangial distribution (Fig. 2). Glomerular fluorescence declined between days 1 and 7, and virtually no staining for ferritin was detectable by or after day 14. Little or no fluorescence was noted in the renal interstitium, including the periglomerular regions, or in tubular cells or lumina. Renal immunofluorescence staining for rat IgG showed no or only mild focal-segmental glomerular deposits which appeared strongest on day 7 (trace to 2+).

Light microscopy. Examination of paraffin-embedded tissue sections stained for iron revealed a different time course (Fig. 1**C**), in that the onset of positive glomerular staining was much more gradual than that found for ferritin fluorescence, whereas complete glomerular clearance of iron was not demonstrable within the 4-week study period. The positive staining was always granular and occurred in a mesangial distribution, also involving stalk and lacis area (Fig. 3). Starting at 3 hr after ferritin injection, there were iron-containing cells in scattered areas of the cortical and medullary interstitium. Iron staining of

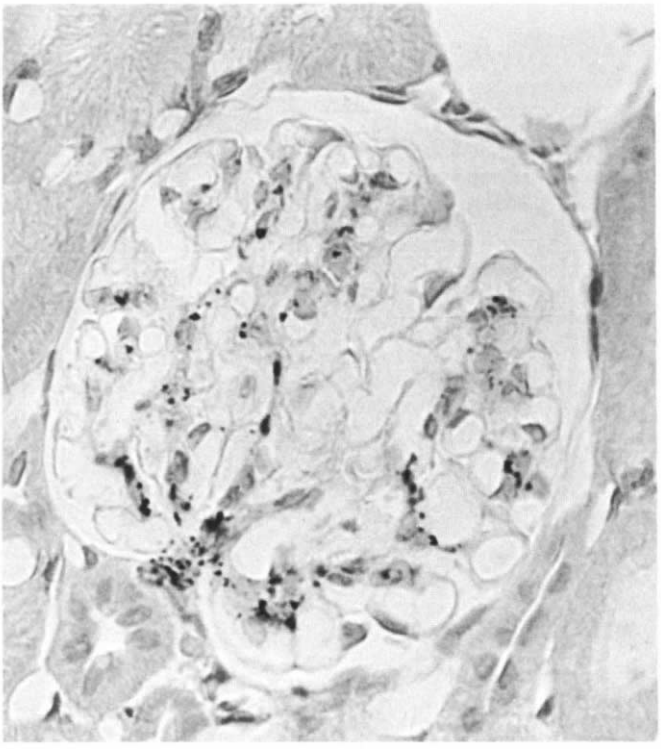

Fig. 3. *Iron stain of renal tissue of a rat 14 days after ferritin injection.* Glomerulus shows glomerular iron deposits *(dark granules)* in a mesangial distribution involving the tuft periphery, stalk, and lacis area. No iron is seen in the perihilar interstitium or the macula densa cells. (×650)

the glomerular stalk area became more prominent with time, but no further accumulation of iron was appreciated in the lacis area or in the perihilar interstitium. Adjacent cells of the distal tubule or of the ascending limb of Henle's loop as well as macula densa cells, identified in serial sections, did not show staining for iron. Occasionally, proximal tubular cells stained faintly blue. No other histologic abnormalities, such as glomerular or interstitial hypercellularity or widening of the mesangium, was observed in sections stained with hematoxylin and eosin.

Electron microscopy. Ferritin was seen in the capillary lumen, within endothelial fenestrations, both axially and peripherally, and in the lamina rara interna from 1 min to 3 hr after injection. By 10 min after injection, many ferritin molecules were present in the mesangial matrix where it underlies the attenuated and fenestrated endothelium and where it connects with the lamina rara interna of the peripheral loop (Fig. 4). Tracer particles were first seen in membrane invaginations and cytoplasmic vacuoles of mesangial cells at 30 min (Fig. 5). The matrix became free of ferritin after 1 to 3 days. Ferritin within the mesangial cells appeared in membrane-limited bodies (phagolysosomes) initially as native molecules, showing a characteristic symmetrical hexagonal configuration, and later only as irregularly condensed iron cores (Fig. 6). The time course of glomerular handling of ferritin, as seen by electron microscopy, is summarized schematically in Figure 7. Whereas the large majority of cells in the mesangium showed ferritin uptake, the endothelial and epithelial cells were ordinarily negative. Processing of ferritin in the lacis cells and their surrounding matrix was similar to that found in the intraglomerular mesangium. No marked shift of ferritin from the capillary lumen to the glomerular vascular pole via the mesangium was observed with time. The macula densa cells remained free of ferritin. Blood-borne monocytes with ferritin in many phagolysosomes were occasionally seen in the glomerular capillary lumen and very rarely protruding into or out of the mesangial space. Mesangial hypercellularity was not evident at any time of the study.

Ia-antigen bearing cells. In normal rat kidneys, brightly stained Ia-positive cells were observed in mesangial areas of most glomeruli (Fig. 8). The mean count was 2.1 cells per glomerular section (range 0 to 6). These cells appeared to be located in the center of glomerular lobules or in stalk areas, as judged by simultaneous phase contrast microscopy. They were of irregular shape and showed discontinuous staining for Ia on high magnification. Positive cells were also seen throughout the renal interstitium. In ferritin-treated rats, the counts of Ia-antigen bearing cells increased slightly (Table 1). Using simultaneous phase contrast microscopy, ferritin was detectable in refractile granules in a mesangial distribution, most prominently after day 3. The large majority of ferritin granules was located to Ia-negative cells. The Ia-positive cells displayed a non-uniform pattern regarding localization of ferritin. While some of them were associated closely with ferritin granules, others clearly were not.

Histochemical staining for nonspecific esterase. Rare positive cells were seen in glomerular tufts of untreated rats, the average glomerular count being 0.1 positive cells per glomerular tuft section (range 0 to 1). After injection of ferritin, the average count of esterase-positive cells increased gradually reaching a maximum mean of 1.3 on day 3 (Table 1). The observed esterase activity was inhibited strongly by addition of NaF to the incubation medium. The range of positive cells per tuft was between 0 and 3. Thus, there were many glomerular sections without esterase-positive cells, despite the fact that virtually all glomeruli included cells containing iron. This discrepancy became particularly obvious when sections were first stained for esterase and then superstained for iron (Fig. 9).

Discussion

This study was designed to examine mechanisms involved in the glomerular processing of macromolecular proteins accumulated in the mesangium during the process of glomerular ultrafiltration. Ferritin was chosen as a probe since it allows simultaneous assessment of the fate of the protein shell and the residual iron core. The obtained results confirm and extend the findings of Farquhar and Palade [6] and Farquhar, Wissig, and Palade [16] and of Takamiya et al [18], who showed earlier that ferritin gains access to the mesangial matrix within a few minutes after intravenous injection. Analogous results have been reported by other investigators who used different probes, such as thorotrast, colloidal carbon, iron dextran, and protein aggregates [7–9, 22–24]. This indicates that plasma, including large molecules, freely moves through the attenuated and fenestrated endothelial layer of the glomerular capillary and enters the mesangial space. While it appears as if ferritin enters the mesangium predominantly by a direct route from the lumen to the intercellular mesangial spaces, additional percolation of ferritin from the lamina rara interna in the periphery of the capillary loop into

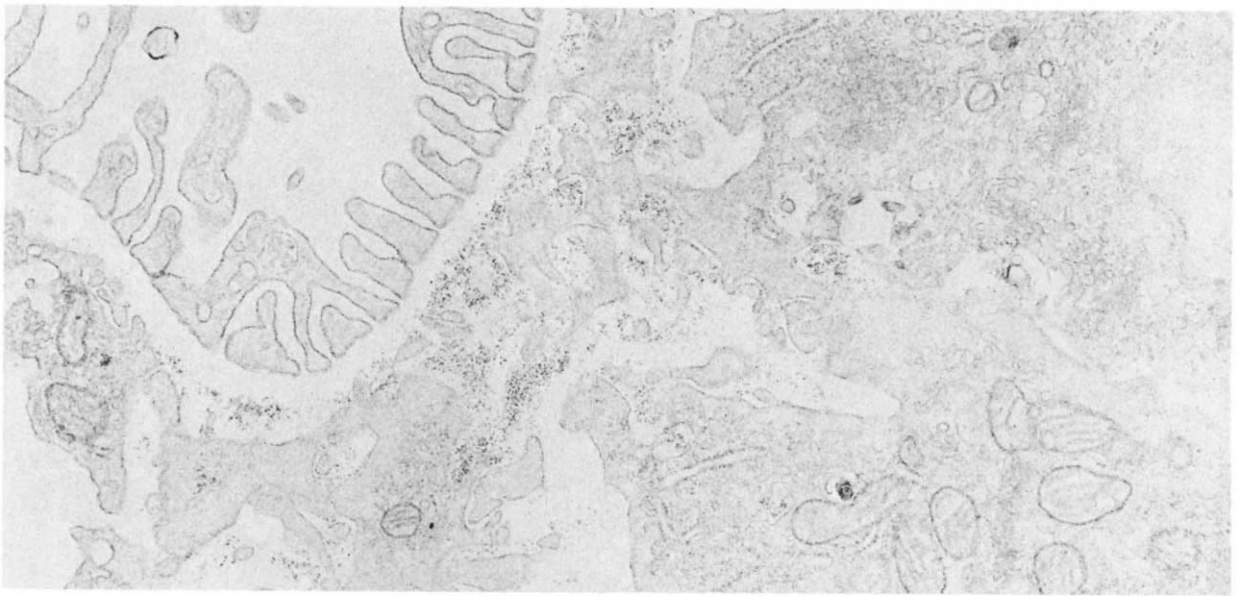

Fig. 4. *Electron micrograph of a glomerulus 10 min after the injection of ferritin.* Ferritin can be seen as fine electron dense symmetrical granules along the lamina rara interna of the basement membrane beneath the endothelium and in the mesangial matrix. (×21,400)

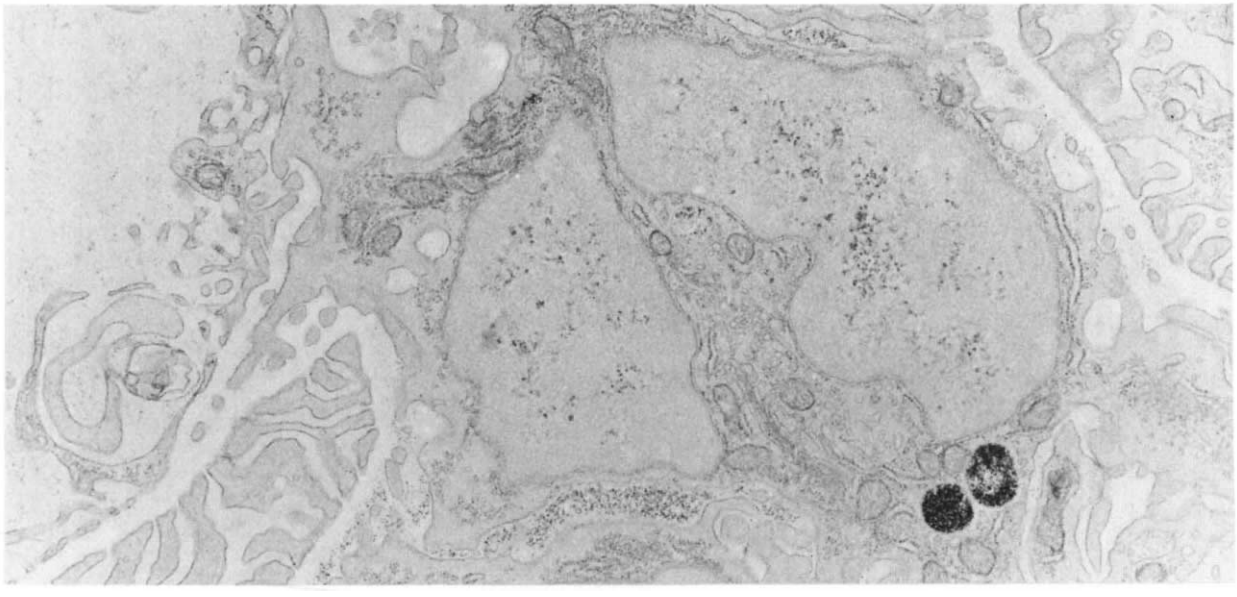

Fig. 5. *Electron micrograph of a glomerulus 3 hr after the injection of ferritin.* Ferritin is no longer present in a subendothelial location. Ferritin is now most prominent in the mesangium, both in the matrix and phagolysosomes of mesangial cells. These mesangial cells do not have numerous lysosomes and other cytoplasmic structures usually seen in blood-borne monocytes. (×16,800)

the mesangium may also occur, as originally suggested by Farquhar and Palade [6]. The driving forces for these movements and their rates of tracer delivery are presently unclear [3]. In this study, the electron-microscopic appearance of ferritin in the subendothelial zones of the peripheral capillary was much more transient than in the mesangium, paralleling the short-lived detection of the tracer in the lumen. While this finding may be partly due to loss of a proportion of soluble ferritin molecules from the lumen and subendothelial space because of tissue handling prior to fixation, it is consistent with the observation of Rennke, Cotran, and Venkatachalam [25] that native anionic ferritin, in contrast to its cationized form, does not accumulate substantially in the peripheral capillary loop of mice.

At present there is incomplete knowledge about the factors which determine whether any given constituent of plasma is retained in the mesangium in appreciable amounts. In the case of heterologous ferritin, both the protein shell as well as the iron component were still present in the mesangium 1 and 3 days after injection, at a time when ferritin was hardly or no longer

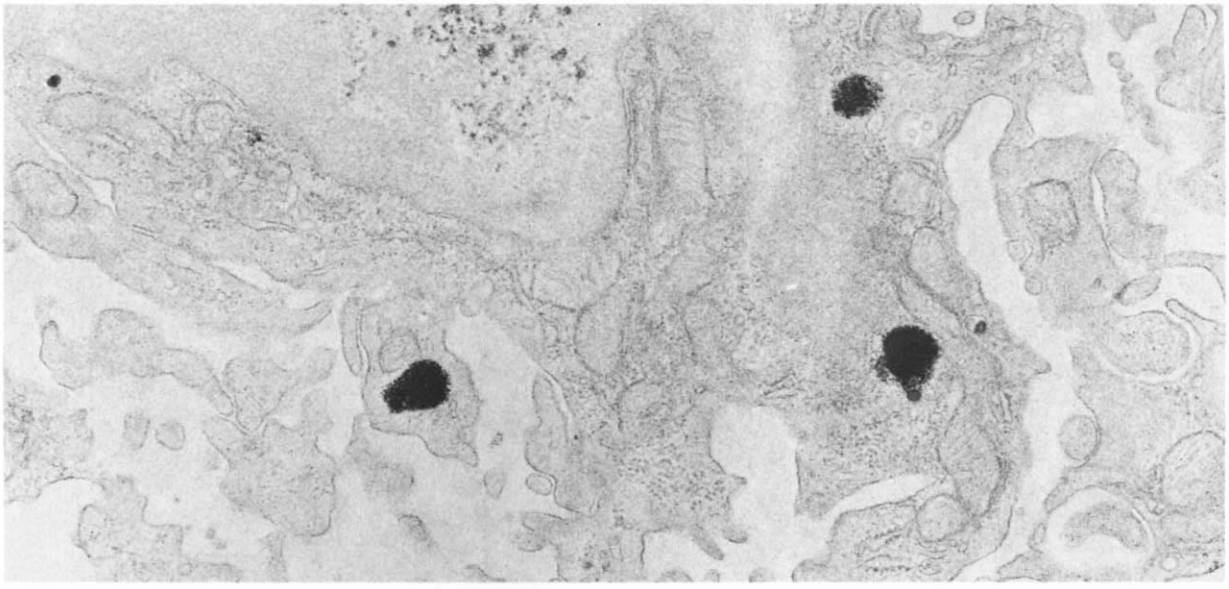

Fig. 6. *Electron micrograph of glomerulus 7 days after injection of ferritin.* Ferritin is no longer present in the matrix and can be found only in phagolysosomes of mesangial cells. Ferritin now appears as condensed electron dense granules representing the tightly packed iron cores of the molecules remaining in the phagolysosomes after degradation of the protein coat. (×25,200)

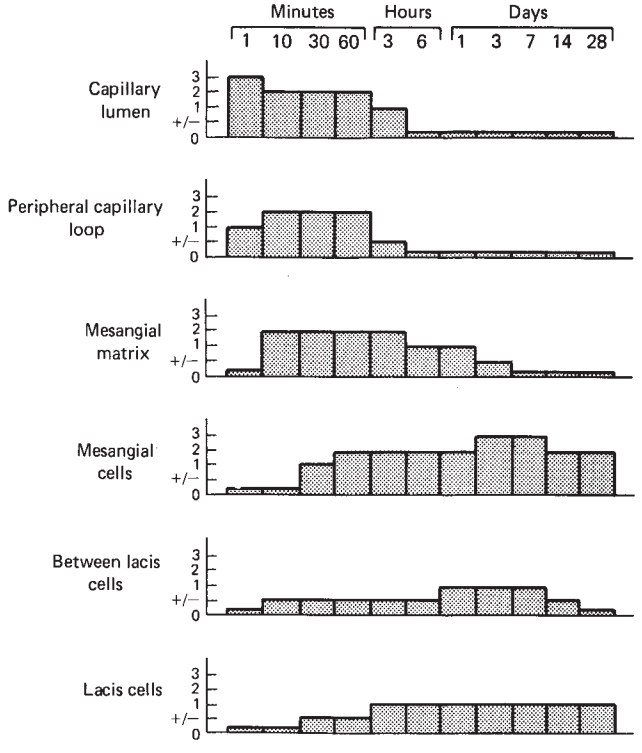

Fig. 7. *Schematic representation of the average amounts of ferritin noted by electron microscopy in different locations of glomeruli from rats sacrificed 1 min to 28 days after ferritin injection.* The results are graded: negative = 0, trace = +/−, moderate = 1, heavy = 2, massive = 3. Each result represents the average obtained from glomeruli of three or four rats.

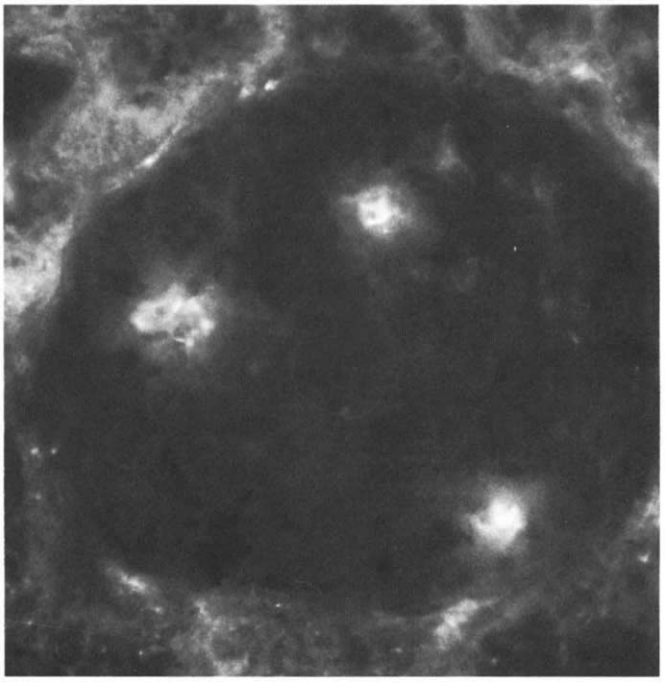

Fig. 8. *Immunofluorescence staining for Ia-antigen bearing cells in renal tissue of a rat. 14 days after ferritin injection.* Ia-positive cells are seen in the glomerulus and in the cortical interstitium. (×650)

detectable in the circulation. This indicates that presence of native ferritin in the mesangium is not only a function of its availability in plasma and that the rate of the mesangial clearing is slower than the rate of entry resulting in retention of this molecule in the mesangium. Takamiya et al [18] have shown that injection of ferritin which is conjugated with human IgG or albumin increases the initial mesangial uptake as well as the persistence of the tracer in the glomerulus. This observation suggests that the mesangial processing of ferritin is modulated by its molecular size and antigenic conformation.

Table 1. Counts of glomerular cells which stain for Ia-antigen and for non-specific esterase[a]

	Control	Time after ferritin injection					
		1 hr	6 hr	1 day	3 days	14 days	28 days
Ia-positive cells							
Mean	2.1	NT	2.3	2.2	NT	2.8	NT
Range	0 to 6		0 to 5	0 to 6		0 to 8	
(*N*)	(8)		(6)	(4)		(4)	
Esterase-positive cells							
Mean	0.1	0.1	0.3	0.6	1.3	1.1	0.2
Range	0 to 1	0 to 1	0 to 2	0 to 2	0 to 3	0 to 3	0 to 2
(*N*)	(12)	(6)	(6)	(4)	(6)	(6)	(4)

[a] Values represent counts of positive cells per glomerular section. Data were obtained from 50 glomeruli in the examined renal sections. *N* denotes number of studied animals; NT, not tested.

The electron microscopic findings at days 3 and 7 show that most or all of the ferritin found in the mesangium, after complete clearance from the circulation, was restricted to intracellular locations. Since glomerular fluorescence staining was still prominent at this time, we conclude that a portion of the protein component of ferritin molecules is antigenically intact when being taken up by cells in the mesangium. This finding is consistent with immunoelectronmicroscopic results of Lee and Vernier [7] who observed in mice that a small fraction of injected human albumin aggregates was still demonstrable inside mesangial cells. Using an analogous technique to detect the presence and fate of internalized proteins within cells, Mancilla-Jimenez et al [9] also demonstrated prominent in vivo endocytosis of heat-aggregated antiperoxidase IgG by mesangial cells of rats. Accordingly, one way by which macromolecular proteins are removed from the matrix is by uptake into cells located in the mesangium. Further intracellular digestion of apoferritin is indicated in our study by subsequent complete loss of staining for the intact protein as well as by the intraphagolysosomal changes of the typical ultrastructural image of ferritin molecules which become more tightly packed with time, showing dense and irregular accumulation of the iron cores. These results emphasize that proteinaceous macromolecules which are taken up by cells and undergo partial degradation may escape detection by routine immunohistologic techniques.

Diverging results and interpretations exist with regard to the type of cell responsible for most of the uptake and disposal of mesangial probes. Presently, there are at least three types of actively endocytic cells which have been described to be present in the mesangium.[1] They are *(1)* the intrinsic mesangial cell, *(2)* the Ia-antigen bearing, bone-marrow derived cell which is resident in the mesangium, and *(3)* scavenging monocytes which have infiltrated the mesangium after glomerular deposition of the probe.

Our finding of Ia-positive cells in glomeruli of healthy rats confirms the reports of other investigators demonstrating that these cells constitute a special subpopulation of cells which are resident in the mesangium [4, 5, 27]. Such Ia-positive cells are thought to be bone-marrow derived cells which are capable of presenting antigens to lymphocytes and, thus, resemble Ia-bearing dendritic cells in the spleen and the interstitium of other organs [4, 27]. While the potential importance of Ia-positive cells for the initiation of an immune reaction in the glomerulus is obvious, their endocytic capacities are presently unclear. Schreiner et al [4] studied glomerular cell preparations obtained after enzyme treatment of rat glomeruli and found a fraction of the Ia-positive cells to be markedly endocytic for heterologous IgG aggregates in vivo and for latex beads in vitro. Hart and Fabre [27] reported that colloidal carbon injected into rats did not localize in appreciable amounts to Ia-positive cells in glomeruli examined in tissue sections. Using simultaneous immunofluorescence and phase contrast microscopy of renal tissue, we noted that only a proportion of Ia-positive was clearly endocytic for ferritin. It was also evident that the bulk of the glomerular ferritin deposits was taken up by cells which did not stain for Ia antigens. There may be several reasons for the apparent discrepancy between our findings and those of Schreiner et al [4]: First, it is possible that the employed tracers, differing in nature, size and biologic activity may cause different endocytic responses of Ia-positive cells. Second, the Ia-positive fraction of all glomerular cells obtained by the isolation procedure used by these investigators was markedly higher than that found in whole glomeruli. Thus, the phagocytic contribution of Ia-positive cells may appear more prominent in vitro. Nevertheless, a substantial fraction of their Ia-positive cells were not phagocytic and many phagocytic cells were Ia-negative. The combined available information, therefore, indicates that these cells are not homogenous as far as their endocytic properties are concerned. Another variable is the expression of Ia-antigen on a given cell, which may be altered by a variety of environmental and immunologic factors. For example, Ia-positivity of murine macrophages was shown to be induced by various immune stimuli and phagocytosis [28, 29]. Clearly, further studies are required to fully analyze the characteristics of Ia-antigen bearing cells in the glomerulus and their precise role in the mesangial handling of different exogenous and endogenous glomerular deposits.

In view of the widespread intracellular uptake of ferritin in the mesangium, our finding of only occasional esterase-positive monocytes would argue against the possibility that transient blood-borne monocytes contribute greatly to the disposal of

[1] According to Silverstein, Steinman, and Cohn [26], endocytosis denotes the uptake of molecules from the cell's environment via plasma-membrane-derived vesicles and vacuoles. Although most eukaryotic cells demonstrate endocytosis, it is most prominent in leucocytes and macrophages. Both soluble (pinocytosis) and particulate (phagocytosis) macromolecules may be internalized.

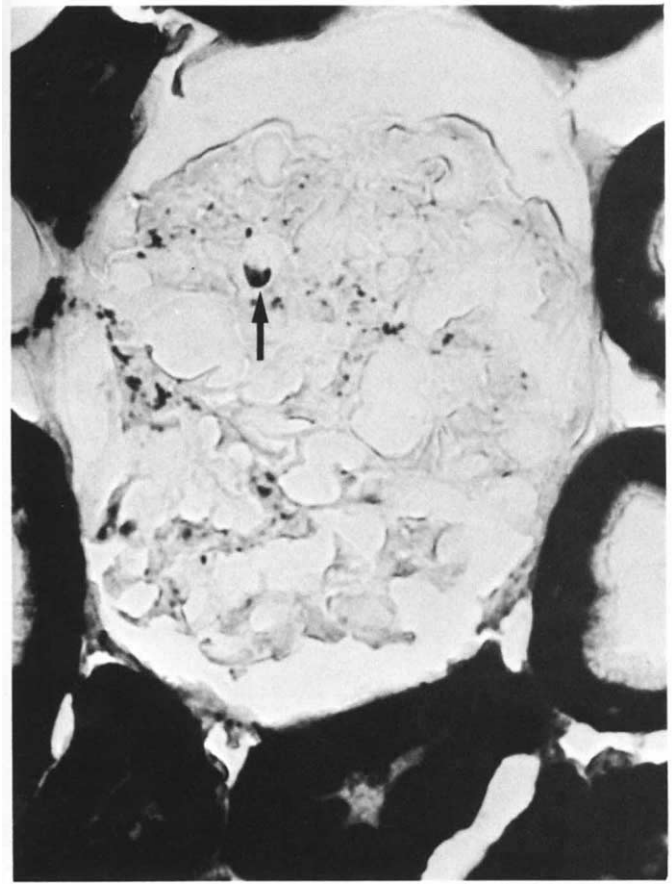

Fig. 9. *Renal tissue of a rat, 7 days after ferritin injection, stained for nonspecific esterase and superstained for iron.* Glomerulus shows multiple glomerular iron deposits *(small granules)* and one esterase positive cell *(arrow)*. Proximal tubular cells show strong staining for esterase. (×650)

intramesangial ferritin. The great majority of circulating mammalian monocytes stain positively for nonspecific esterase [30, 31], and it seems unlikely that glomerular involvement of blood-derived phagocytes would be restricted to esterase-negative monocytes, thus, escaping histochemical detection. Furthermore, the electron microscopic observations revealed only rare cells with typical ultrastructural features of monocytes, such as an abundance of lysosomes. Absence of a sizeable monocytic involvement was also reported for the mesangial processing of a variety of exogenous probes, including protein aggregates [7, 9] and carbon particles [8], as well as for the removal of certain endogenous immune complexes in experimental glomerulonephritis [12, 13]. Similarly, various human glomerular diseases with predominantly or exclusively mesangial deposition of endogenous immune proteins lack a prominent congregation of monocytes in glomeruli [14, 15]. Divergent observations were reported by Striker, Mannik, and Tung [10] who used a complex model of Chediak-Higashi mice involving bone marrow transplantation to examine the mesangial handling of protein aggregates. The findings made by this group suggested a prominent participation of blood-borne monocytes in the mesangial uptake of the used probe. A similarly conspicuous involvement of monocytes-macrophages was observed recently in this laboratory for the mesangial uptake of the synthetic polysaccharide, polyvinyl alcohol [11]. Taken together, the presented findings indicate that the contribution of monocytes-macrophages to the removal of mesangial deposits depends heavily upon the nature and biologic activity of the deposited macromolecule. We conclude that, analogous to ferritin, many types of exogenous and endogenous macromolecules are disposed in the mesangium by intrinsic mesangial cells. Recruitment of scavenging monocytes-macrophages occurs when certain deposits generate chemotactic stimuli or other mediators which induce the infiltration of the mesangium by inflammatory cells.

The present results do not answer the questions of what fraction of ferritin molecules which have entered the mesangial matrix is digested in mesangial cells, and what the fate of the remaining portion might be. For example, it remains unclear whether degradation of ferritin may also occur extracellularly in the matrix, followed by cellular uptake or egress of ferritin fragments. Our sequential observations on the localization of ferritin in the glomerulus make it unlikely that major amounts of the probe could have percolated through the mesangial matrix of the glomerular stalk beyond the lacis area at the vascular pole into the adjacent interstitium or to tubular cells. Such pathways have been suggested by several authors to be operative in mice and rats when probes such as colloidal carbon [23], iron dextran [24], and protein aggregates [7] were used. In this study, iron-stain positive cells, indicating actively phagocytic renal histiocytes, were the only evidence for presence of ferritin in the interstitium. These phagocytes, which effectively degraded ferritin as judged by the lack of prominent immunofluorescence staining for apoferritin antigen, were scattered throughout cortex and medulla, not suggestive of a glomerular origin of the internalized ferritin. Studying rats immunized with repeated ferritin injections, Keane and Raij [12] also failed to detect passage of ferritin or of endogenous IgG and C3 from the mesangium into the interstitium or tubules. The reasons for the discrepancy of these findings regarding the egress of different macromolecules from the mesangium into the cortical interstitium are presently unclear. More extensive morphometric studies are required to determine the kinetics of tracer removal from the matrix in the lacis area and to clarify whether macromolecules found in the adjacent interstitium are, indeed, derived from the mesangium or originate from the peritubular capillaries supplying this region.

In agreement with previous findings of Farquhar and Palade [6] and Farquhar, Wissig, and Palade [16], the initial localization of ferritin molecules was most prominent in the mesangial matrix where it underlies the paramesangial GBM (Figs. 4 and 5). This could either reflect ongoing filtration of plasma constituents which entered the mesangium, with ferritin molecules as residues, or it could be due to undefined physicochemical factors which favor the appearance of ferritin in the mesangial space facing the GBM. Since the clearance of ferritin from this area was not accompanied by its increasing localization within the layers of the GBM, we conclude that gradual passage of ferritin or iron cores through the GBM does not take place in appreciable amounts. The observation that ferritin was cleared from all areas of the mesangial matrix within a similar time interval indicates that the areas underlying the GBM are readily accessible to either uptake by adjacent cells or drainage. By exclusion, one could speculate that removal of intact ferritin

from the mesangial space, bypassing mesangial cells, may take a route which would have returned ferritin back to the blood, possibly further "downstream" toward the efferent glomerular arteriole, as has been originally postulated by Latta and Maunsbach [22]. At present the available techniques do not permit measurement of the factors governing such processes, for example, physical forces which determine the movement of plasma constituents across the mesangial space.

In summary, the present findings show that the rat glomerulus disposes of heterologous native ferritin by uptake by mesangial cells followed by degradation of apoferritin and storage of the iron cores in phagolysosomes. The contributions of Ia-antigen-bearing resident mesangial cells and of infiltrating monocytes to the disposal of ferritin appear small. While the fraction of ferritin which bypasses the mesangial cells and is drained from the mesangium remains undetermined, there is no evidence that this takes a route from the glomerular tuft via the vascular pole to the adjacent cortical interstitium or tubules.

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